Marine biofouling: a sticky problem

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Organisms that colonise underwater surfaces, such as barnacle larvae and spores of algae, use a diverse array of biological 'glues' to provide both temporary and more permanent adhesion. The practical consequence of colonisation by these organisms is biofouling – something that has plagued mariners for years – causing increased drag and, in extreme cases, corrosion. Might there be a biological solution to this biological problem?

Marine biofouling is caused by the adhesion of barnacles, macroalgae and microbial slimes. It is a worldwide problem in marine systems, costing the US Navy alone an estimated $1 billion per annum. On ships' hulls, biofouling results in an increase in roughness, which in turn leads to an increase in hydrodynamic drag as the vessel moves through water. Increased fuel consumption, hull cleaning, paint removal and repainting, and associated environmental compliance measures all contribute to the costs of biofouling.

New, effective, and environmentally compatible options are needed to control fouling. An active research front is aimed at understanding how adhesives, produced by fouling organisms, interact with surfaces, so that coatings may be designed in a rational way to inhibit this process. Antifouling paints have a profound effect on the environment, and research on bioadhesives may contribute to the development of environmentally benign fouling control.

Biofouling: micro- and macro-fouling

Within minutes of immersing a clean surface in water it adsorbs a molecular 'conditioning' film, consisting of dissolved organic material. Bacteria colonise within hours, as may unicellular algae and cyanobacteria (blue-green algae). These early small colonisers form a biofilm: an assemblage of attached cells sometimes referred to as 'microfouling' or 'slime'.

Diatoms (Figure 1) are unicellular algae in which the protoplast is enclosed in an elaborately ornamented silica case (the frustule) composed of overlapping halves or 'valves'. Diatoms predominate in biofilms that adhere to certain types of antifouling coating. They adhere to surfaces by secreting sticky extracellular polymeric substances (EPS) via an elongate slit, the raphe, in one or both valves. (EPS also provide the mechanism for the diatoms' 'gliding' motility.) Attached cells divide, rapidly giving rise to colonies that eventually coalesce to form a compact biofilm, which may achieve 500 µm in thickness.

A macrofouling community (consisting of either 'soft fouling' or 'hard fouling') may develop and overgrow the microfouling. Soft fouling comprises algae and invertebrates, such as soft corals, sponges, anemones, tunicates and hydroids; whilst hard fouling comprises invertebrates such as barnacles, mussels and tubeworms. The specific organisms that develop in a fouling community depend on the substratum, geographical location, the season, and

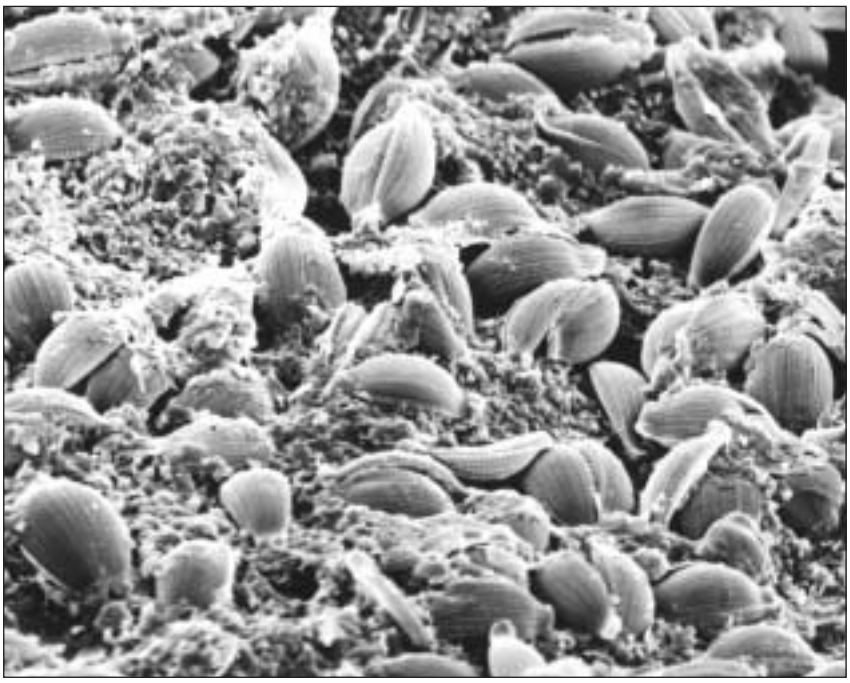

Figure 1. Scanning electron micrograph of a diatom slime growing on a copper antifouling paint. The diatoms are species of Amphora.

factors such as competition and predation. Fouling is a highly dynamic process.

Adhesion of fouling organisms

Fouling control is essentially a problem of managing adhesion. One of the fascinating areas of pure and applied research concerns the nature and properties of the underwater glues. Nature, unlike technology, has very effectively conquered the limitations of sticking to wet surfaces! The interaction between an adhesive material and the substrate involves two main steps: a) wetting of the substrate by the adhesive, and b) curing of the adhesive. The wetting process determines the actual area of contact between the adhesive and the substrate. It also has an important role in determining the interaction force between the adhesive and the substrate. The curing process determines the microstructure of the solid film, thus influencing both the mechanical properties and the adhesive strength.

Representatives of all the phyla of marine biofoulers use sticky materials with permanent or temporary adhesive capabilities, but adhesion mechanisms and detailed molecular characteristics of the glues are largely unknown. The two exceptions to this are the glues produced by **adult** marine invertebrates. The protein glues of the blue mussel have been extensively characterised and are members of a dihydroxyphenylalanine (DOPA)-rich family of polypeptides, which cross-link through an oxidative phenolic tanning type process. The second example, the cement produced by mature adult barnacles, appears to consist of a complex of hydrophobic proteins that are unrelated to blue mussel proteins, crosslinked via cysteine residues.

Mussels produce threads to attach themselves to solid surfaces in the inter-tidal zone. These 'byssus' threads, secreted by the mussel foot are effectively biocomposites of collagen fibres embedded in a proteinaceous matrix. Mefp-1 is the best characterised of the polyphenolic foot adhesive proteins. It consists almost entirely of repeating tandem decapeptide and hexapeptide sequences, with a high level of post-translational hydroxylation of tyrosine and proline to DOPA and hydroxyproline respectively. On oxidation to o-quinone the DOPA molecules become highly reactive, forming strong bonds with metal ions, including those presented at surfaces, and inducing cross-links within the protein itself and with other proteins in the biocomposite. Current research is aimed at identifying the structural elements responsible for its remarkable adhesive and cohesive properties, and a promising approach is to use peptide analogues of the repeat motifs. These biomimetic materials have equivalent properties to the native molecule and their simpler structure enables the biochemical identification of specific group-surface interactions that contribute to the adhesive properties.

Dispersal stages

Arguably more relevant to the problem of biofouling and its control are the dispersal stages of organisms, rather than the adult forms. Larvae of invertebrates and spores of algae need to quickly find and bind to a surface in order to complete their life history. This adhesion process takes place within seconds, under water, to a wide range of substrates, over a wide range of temperatures and salinities, and in conditions of turbulence. In certain cases the adhesion is effectively permanent; in other cases adhesion needs to be reversible as the organism moves around on a surface to find the most appropriate settlement site. This phase of initial, or first-contact, adhesion to a substrate is shown by diverse single- and multi-cellular fouling organisms and has been termed 'the first kiss'. An understanding of the cellular and molecular processes and materials involved underpins a lot of contemporary basic research in marine fouling.

'First-kiss' in *Enteromorpha*

The green alga *Enteromorpha* is known to most of us as the slippery grass-like plant that covers rocks in the intertidal zone. It is the major macrofouling alga and forms the focus of research in our laboratory. *Enteromorpha* colonises new surfaces through the production of vast quantities of microscopic motile spores (Figure 2a). Swimming spores attach rapidly once they have 'detected' a suitable surface for settlement, resulting in firm attachment to the substratum (Figure 2b). Spore germination often occurs within a few hours, giving rise to germlings. These are attached to the substratum by adhesive that is secreted by the rhizoids.

'First-kiss' adhesion in *Enteromorpha* involves a pattern of behaviour on the part of the swimming spore during which it is responsive to a range of cues. This is followed by an irreversible commitment to adhesion involving withdrawal of flagella and the secretion of a powerful adhesive (Figure 3). A number of 'cues' are involved in attracting spores to a particular surface, on which to settle and attach. Negative phototaxis guides zoospores and zygotes to areas of low light, although diffusible chemical cues released from surface-associated organisms such as bacteria may also guide the swimming spores towards a suitable surface. We have also shown that settlement is strongly influenced by a number of surface properties including wettability and microtopography.

Once a suitable area for settlement is located, the spore

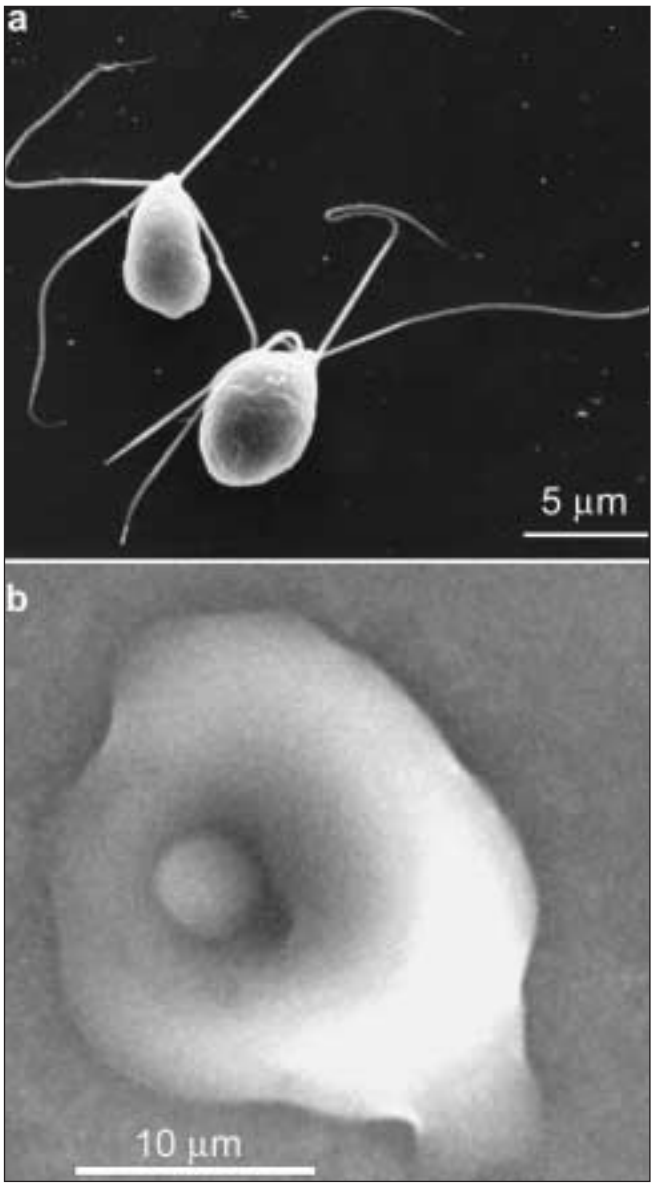

Figure 2. Swimming and settled zoospores of Enteromorpha. *a) is a conventional SEM of swimming, quadriflagellate zoospores. b) is an image of a settled, adhered zoospore taken in an Environmental Scanning Electron Microscope (ESEM) without any fixation or substantial dehydration. It shows the original spore surrounded by the pad of secreted adhesive. JAC acknowledges the support of Professor Athene Donald and Dr Frank Baker at the Cavendish Laboratory, Cambridge in carrying out the ESEM studies.*

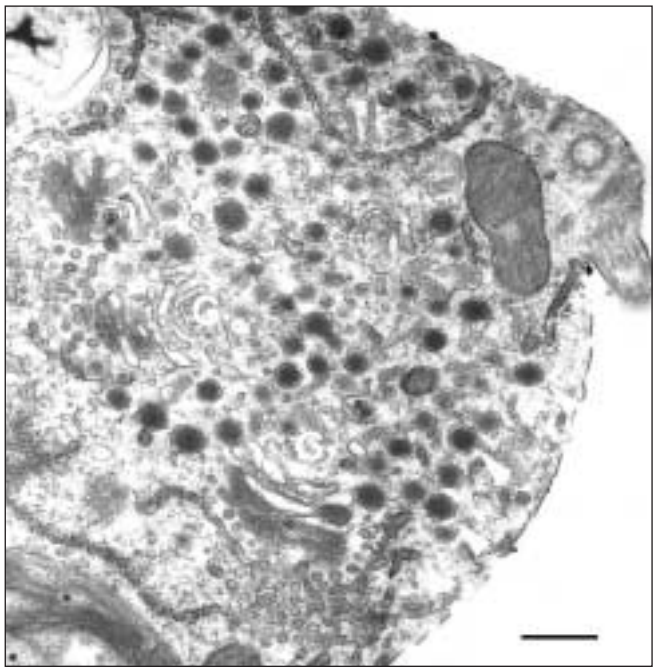

Figure 4. Electron micrograph of a section through the apical region of a swimming Enteromorpha zoospore showing the extensive adhesive vesicles with electron-opaque deposits of the primary adhesive (arrows). The vesicles are formed from Golgi bodies (G). Image courtesy of Professor L V Evans. (Scale bar 500 nm)

then secretes a glycoprotein adhesive by exocytosis of the con-tents of mem-brane-bound cytoplasmic 'adhesive' vesicles, formed from the Golgi appa-ratus (Figure 4). The glue forms an ad-hesive pad surrounding the spore. We have recent-ly used a re-latively new technique, atomic force microscopy (AFM), to ex-amine some of the properties of the spore glue.

Atomic Force Microscopy (AFM) is a useful investigative tool that provides three-dimensional images of surface topography of biological specimens in ambient liquid or gas environments. Unlike electron microscopes, samples do not need to be fixed, dehydrated, coated or frozen. The instrument uses neither optical nor electronic lenses. Instead, it relies upon sensitive laser detection of deflections to a small cantilever-mounted tip, which occur in response to intermolecular forces. The tip is raster-scanned across a surface.

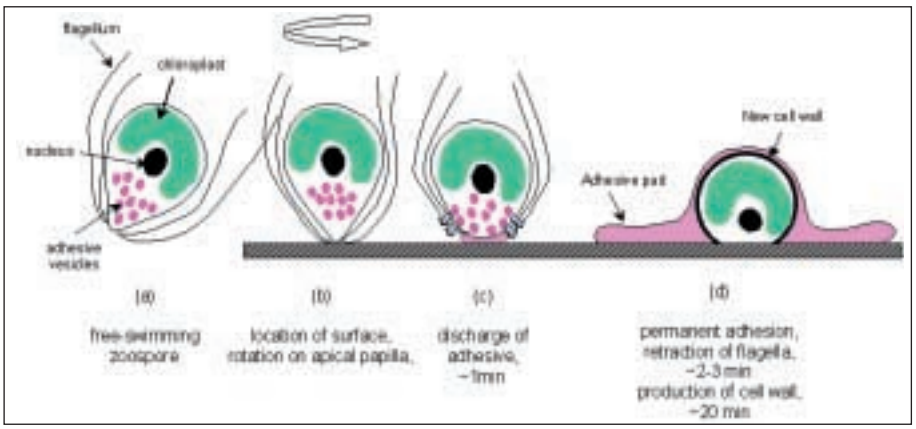

Figure 3. Cartoon representation of the stages involved in Enteromorpha *zoospore settlement and adhesion. During the surface sensing phase the swimming spore undergoes a characteristic pre-settlement behaviour that involves a switch from random swimming to a 'searching' pattern of exploration close to the substratum. The spore appears to become temporarily adhered to the substratum as it spins like a 'top' on its apical dome, the flagella acting as propellers. The spore then commits itself to irreversible permanent adhesion involving the secretion of a powerful adhesive from pre-formed cytoplasmic vesicles. A cell wall is formed later.*

AFM can also be used to measure visco-elastic properties of materials, such as adhesive strength, hardness and elasticity. Recently, we used AFM to make such measurements on the Enteromorpha adhesive pad in its hydrated state. Freshly released adhesive has an **adhesion strength** of approximately 500 mN m^{-1} (indicating a very sticky material) and its compressibility is similar to a 20% solution of gelatin. Within minutes of release the adhesive undergoes a progressive 'curing' process, presumably by cross-linking, becoming less sticky and more compressible and assuming a consistency similar to natural rubber.

Figure 5. The cyprid larva of barnacle, Balanus amphitrite, showing the two antennular appendages by which the larva 'walks' as it explores a surface before firmly adhering and metamorphosing into an adult barnacle. Photo courtesy of Dr Tony Clare.

'First-kiss' in barnacles

'First-kiss' adhesion behaviour in barnacles is somewhat different. Barnacle larvae swim around freely in the water column but in order to complete the transition to adult life, the 'cyprid' form of barnacle larvae (Figure 5) must attach to a hard substrate. Cyprids explore a surface by 'walking' using a pair of attachment organs, or 'antennules', which secrete an adhesive from unicellular glands. In this exploratory phase, the cyprids have to be capable of detaching, leaving behind blobs of temporary adhesive 'footprints'. The temporary adhesive does not disperse in water; it is resistant to biodegradation, and also operates as a signalling molecule to induce the settlement of additional cyprids. Otherwise, its physical properties (whether it has any structure, whether it cures, etc) are unknown.

After selection of an appropriate site on which to settle, the cyprid stands on its head and releases proteinaceous cement onto the paired antennules. Initially fluid, this permanent cyprid cement flows around and embeds the attachment organs, curing within one to three hours to form a discrete matrix. The firmly attached organism subsequently metamorphoses into the calcified adult barnacle (Figure 6).

Figure 6. Adult barnacles on a fouled surface: firm attachment is achieved by the calcified baseplates, portions of which remain when the shells are detached. Photo courtesy of Dr Tony Clare.

Methods of fouling control

Mariners from ancient times were aware of the problems resulting from boring and other fouling organisms. The ancient Phoenicians and Carthaginians were said to have used pitch and possibly copper sheathing on their ships' bottoms. The Greeks and Romans both used lead sheathing, which the Romans secured by copper nails. Copper has been in general use by the British Navy since 1780. From these early beginnings, antifouling paints incorporating copper salts developed. Copper binds to sulphur-containing cell constituents, leading to a variety of responses associated with heavy metal toxicity.

The major types of toxic anti-fouling paints in use today are soluble matrix paints, also known as conventional paints, ablative paints (modern versions of conventional paints) and self-polishing systems. The majority of antifouling paints are pigmented with copper, usually as cuprous oxide (Cu_2O).

The *self-polishing copolymer* (SPC) paints, introduced in 1974, were so called to indicate the 'polishing' effect as the polymer dissolves away during normal vessel operation, releasing tributyltin (TBT). TBT kills settling fouling organisms and, at the same time, the surface becomes smoother. Being very lipid soluble, it is rapidly taken up by cells, where it inhibits energy transfer processes in respiration and photosynthesis. The SPC system was extremely successful, but TBT was shown to effect non-target organisms, including a number of shellfish, at levels much lower than ever envisaged. The most sensitive invertebrate species, the dog whelk, *Nucella lapillus,* exhibits imposex (imposition of male sexual characters on the female) at concentrations below 1 ng l^{-1}, and its disappearance from rocky shores in areas of high boating activity has been attributed to the presence of TBT from antifouling paints.

TBT is now prohibited in many parts of the world and it is anticipated that the International Maritime Organisation (IMO) will impose a worldwide ban on the use of TBT-containing paints on any type of vessel from January 2003. Furthermore, the discharge of copper from antifouling paints is currently under scrutiny, especially in California.

The impact of TBT on the aquatic environment has also led to an increase in the regulations affecting the use of all other antifouling biocides, and only a few are now employed. Most commonly used are Sea-Nine 211 (an isothiazolone), zinc pyrithione (an anti-dandruff fungicide) and Irgarol 1051 (a triazine herbicide). All of these compounds are used mainly as co-biocides to copper, especially to increase efficacy against algae. A new self-polishing system based on copper acrylate is reported to provide control of fouling comparable to the TBT-containing SPC paints.

In the current climate, registration of new active ingredients for use in antifouling paints is a very costly and protracted business. Increasing regulatory, environmental and product safety standards have all increased the cost and time required to develop a new antifoulant. Thus, there is intense research activity to seek novel, environmentally benign, methods of fouling control.

Fouling-release coatings

Silicone fouling release coatings have been developed as an alternative to biocide-containing paints. They function by minimising the adhesion strength of attached organisms,

which are removed as the vessel moves through the water or by special cleaning procedures. Data on the strength by which barnacles adhere to silicones can be used to predict the ship-operating conditions required for self-cleaning. Macroalgae and some hard foulers such as barnacles detach relatively easily from such surfaces, but diatom slimes, oysters and tubeworms are attached tenaciously and are not easily removed, even at high speed. Silicone elastomers are also expensive and prone to tearing, so are only employed at the present time for specific applications, such as on high speed vessels (where release of biofouling is effective) and in locations where toxic paints are prohibited. Research is underway to improve our understanding of the interactions between biological glues and fouling release surfaces, with the goal of improving performance and durability.

Novel technologies

Most approaches to novel control methods have focused on the search for compounds that might repel or inhibit the adhesion of fouling organisms. It has been noted that many organisms in the sea remain free from fouling. Active natural compounds have been extracted from many types of organisms including bacteria, corals, sponges, seaweeds and sea grasses, although some have proved to be highly toxic.

In today's climate, a potential fouling control agent must have a good mammalian and ecotoxicological profile. One such compound is zosteric acid, a sulphoxy phenolic acid derived from eelgrass (*Zostera marina*), which inhibits the accumulation of fouling by interfering with adhesion. Another approach is to synthesise 'non-stick' polymers that have the same surface properties as those organisms that remain foulant free. The range of potential chemistries is large, from the hydrophilic proteoglycans of the sea urchin to the hydrophobic glycoproteins of porpoises and killer whales. Increased molecular understanding of how marine bioadhesives work should also open up novel technologies, such as the incorporation into paints of compounds that inhibit adhesive cross-linking.

Conclusions

The impact of TBT antifouling paints on the environment has resulted in a new public awareness regarding methods of fouling control. This, together with the resulting increased costs associated with environmental and health compliance has meant that the development of new, persistent toxic compounds to control fouling is neither socially acceptable nor economically viable. In the short-term, copper-based antifouling paints will be used on the majority of vessels. But, research is now focusing on the development and evaluation of a variety of benign antifouling strategies. Such strategies are extremely varied and necessitate a multidisciplinary approach to research. Probably the most promising approach is to interfere with bioadhesion of the fouling organisms. In order to do this, a better understanding is required of the molecular and materials properties of the adhesives, and the cellular processes involved in their synthesis and secretion.

Acknowledgements

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References

Two special issues of the journal *Biofouling* (volumes 15 and 16, both published in 2000) contain a selection of research publications from recent international symposia in the area of biofouling and adhesion.

Websites

http://www.biosciences.bham.ac.uk/labs/callowj/ent
Contains information about the algal biofouling research programme in the authors' laboratory

http://www.biosciences.bham.ac.uk/external/biofoulnet
This site was prepared for the NERC Marine Biofouling Thematic Programme and contains information relevant to the general biofouling theme, as well as specific information about that programme.

http://www1.npm.ac.uk/set98/set97/dogwhelk.htm
Contains information on the effect of TBT on dogwhelks.

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